First observation of intersex in barbel *Barbus callensis* (Valenciennes, 1842) collected in Soummam River (Algeria)

by

Hafsa DJOUDAD-KADJI* (1, 2), Sihem BENSLIMANE (1), Christine CHEVALIER (2),
Belaïd KADJI (1), Jean-Marie EXBRAYAT (2) & Mokrane IGUER-OUADA (1)

ABSTRACT. - The presence of intersexuality in a gonochoric species *Barbus callensis* (Valenciennes, 1842) collected between March and October 2010 in the Soummam River, Algeria, is reported using anatomical and histological investigations. A total of 16 out of 700 dissected specimens (2.28%) were macroscopically classified as intersex, i.e. characterized by the simultaneous presence of both male and female gonads in the same individual. Three different anatomical forms were observed: (1) two gonads with an ovary and a testis clearly individualized (n = 2), (2) four gonads with distinguished pairs of ovaries and testes (n = 8), and (3) four gonads, in which each ovary was completely linked to a testis (n = 6). Different portions of the gonads representing the three macroscopic forms of intersex were studied microscopically using Mayer's haemalun-eosin and modified azan staining techniques. Histological analysis revealed the simultaneous presence of female and male gametes in each analysed gonad. In the gonads that appeared as ovaries, a majority of female tissue with oocytes at different developmental stages was observed, with cells reaching the final stage of maturation. Within the female tissue, different male germ cells were observed, including maturated spermatozoa. In apparent testes, the same cells were observed, except that male germ cells were predominating. This study reports the first evidence of simultaneous intersexuality in a gonochoric species *Barbus callensis*, revealed at macroscopic and microscopic levels.

RÉSUMÉ. - Première observation d'intersexualité chez le barbeau *Barbus callensis* (Valenciennes, 1842) collecté dans l'oued Soummam (Algérie).

Dans le cadre d'une étude portant sur le cycle sexuel de *Barbus callensis* (Valenciennes, 1842) de l'oued Soummam, il a été constaté, chez certains individus, des cas d'hermaphrodisme synchrone. Les observations macroscopiques des gonades mettent en évidence 16 cas d'hermaphrodisme parmi les 700 individus examinés, soit 2,28% de l'échantillonnage. L'étude morphologique de l'appareil reproducteur de ces spécimens a permis de distinguer trois types de complexes gonadiques anormaux. Le premier est composé d'un testicule et d'un ovaire (n = 2), le deuxième de deux testicules et deux ovaires séparés (n = 8), et le troisième de deux testicules liés intimement à deux ovaires (n = 6). L'étude histologique de ces gonades a permis de mettre en évidence, au sein des régions femelles, des îlots de cellules sexuelles mâles (spermatocytes et spermatozoïdes) éparpillés entre des follicules ovariens à différents stades de développement. Dans les régions mâles, il a été observé une prédominance du tissu conjonctif dans lequel sont dispersés des spermatocytes, des spermatozoïdes et des ovocytes. Il s'agit de la première signalisation de l'existence d'hermaphrodisme chez *Barbus callensis* aussi bien à l'échelle macroscopique que microscopique.

Key words. - Cyprinidae - *Barbus callensis* intersex - Algeria - Soumman River - Malformation - Histology - Ovotestis - Testis-ovo.

Intersex, in naturally gonochoristic species, is defined as the presence of both male and female reproductive features within the same gonad (Barnhoorn *et al*., 2010). At microscopic level, these alterations are characterized when oogenesis occurs in the testes, or, conversely, when spermatogenesis is taking place in ovaries (Charniaux-Cotton, 1975). This sexual abnormality has been recorded in many species throughout the world from different families of fishes, including wild populations of freshwater fish, like roach *Rutilus rutilus* (Linnaeus, 1758) (Jafri and Ensor, 1979; Purdom *et al*., 1994; Jobling *et al*., 1998; Beresford *et al*., 2004) and gudgeon *Gobio gobio* (Linnaeus, 1758) (Van Earle *et al*., 2001). These alterations were also reported in the freshwater bream *Abramis brama* (Linnaeus, 1758) in The Netherlands (Lahr *et al*., 2006) and in tilapia *Oreochromis mossambicus* (Peters, 1852) in South Africa (Barnhoorn *et al*., 2010). These abnormalities were generally attributed to the exposure to environmental contaminants, especially certain hormonally active chemicals. These pollutants, collectively called endocrine disrupting chemicals, are known to mimic or block sexual hormones in fish.

As far as we know, only one publication about a barbel species reported the presence of protogynius hermaphroditism in *Barbus plebejus* (Valenciennes, 1842) captured in a

---

(1) Associated Laboratory in Marine Ecosystems and Aquacultural, Faculty of Life and Nature Sciences, A. Mira University, Bejaia, 06000, Algeria. [sihambenslimane@gmail.com] [kadji_belaid56@yahoo.fr] [imokrane@gmail.com]

(2) University of Lyon, UMRS 449, Laboratory of General Biology, UCLy, Laboratory of Reproduction and Comparative Development, EPHE, Lyon, France. [cchevalier@univ-catholyon.fr] [jmexbrayat@univ-catholyon.fr]

* Corresponding author [kadji_hafsa@yahoo.fr]

Cybium 2012, 36(4): 531-538.

polluted river in Italy (Viganò *et al*., 2001). However, in the latter, only microscopic intersex was observed with feminization features revealed by the presence of female germ cells at the first stage of development, and no maturated oocytes or simultaneous macroscopic presence of ovaries and testes are observed in the same individuals.

Despite a large number of studies concerning *Barbus callensis* (Valenciennes, 1842), cases of hermaphroditism in this species have never been reported so far (Almaça, 1970, 1972; Kraiem, 1986, 1989, 1994, 1997; Chaouachi and Ben Hassine, 1998; Cherghou *et al*., 2002). The present study provides the first report of simultaneous intersex observed in *Barbus callensis* revealed at macroscopic level, with simultaneous presence of ovaries and testes in the same individuals, but also at microscopic level with simultaneous presence of female and male germ cells reaching the final stage of maturation stages in the same gonad.

MATERIALS AND METHODS

Animals

Specimens of *Barbus callensis* (Teleostei, Cyprinidae) of mixed ages and sexes were sampled by fishing rod and seining (1.5 m, 3 mm nominal mesh) between March and October 2010, in the Soummam River in Bejaïa (northeast Algeria). A total of 700 specimens were obtained and were sacrificed with an overdose of tricaine methanesulphonate (MS-222).

Sex was identified according to the presence of male or female gonads. Individuals that had both male and female gonads (total: 16) were classified as intersex.

Meristics studies and age measurement

For each of intersex specimen, we have determined the total length in mm with an accuracy of 1 mm, with assistance of an ichthyometer. Total and gonad weights were measured and expressed in g with an accuracy of 0.1 g.

Six to eight scales of each fish were removed from above the lateral line near the tip of the dorsal fin. Scales were washed using tap water, cleaned and studied as dry mounts. Small-sized scales were mounted between two glass slides and the age was determined by counting the number of annuli (Baglinière *et al*., 2001).

Histological studies

Gonads were prepared for the histological examination by fixing in 10% neutralized formalin solution. Gonads were divided into equal pieces using transverse portions (thickness, 5 mm). The samples were then dehydrated in increasing ethanol concentrations (70 to 95%), cleared in xylene and embedded in paraffin wax. Histological sections of 3 μm thickness were then performed using a Leica RM2025 rotary microtome. Sections were mounted on glass microscope slides, stained with Mayer's haemalun-eosin or modified azan, and mounted with Canada balsam. They were then examined by light microscopy, and photographed using a digital camera.

RESULTS

Meristic measurements

Total length of normal males and females was between 90 and 240 mm and 80 and 282 mm, respectively. The total weight varied between 6.33 and 185.43 g for the males and between 5.72 and 301.20 g for the females. Concerning the gonad weight, the intervals were between 0.02 and 7.60 g for the males and between 0.05 and 20.78 g for the females.

Table I summarizes the meristic measurements of inter-

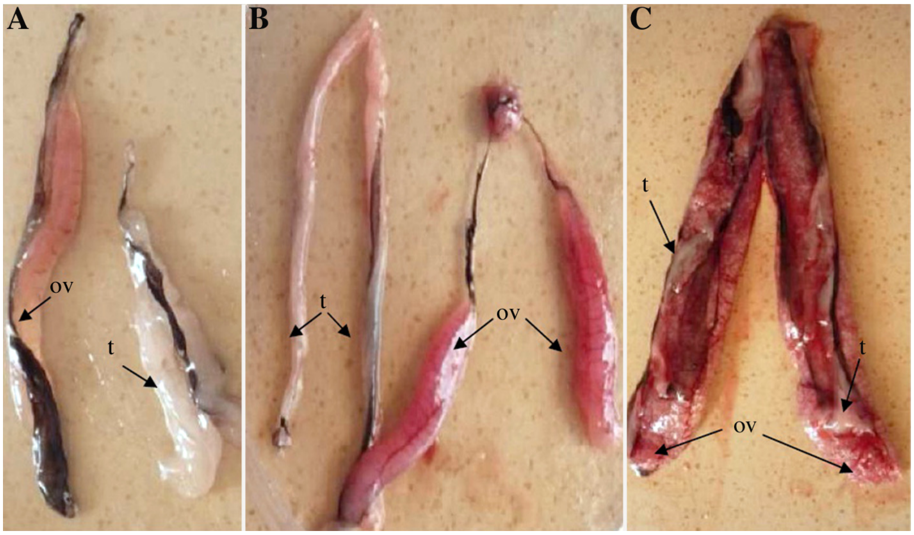

Figure 1. - Gonads of *Barbus callensis* identified as intersex, showing three abnormal forms. **A**: One ovary and one testis clearly separated without oviduct or sperm ducts. **B**: Paired ovaries and oviducts associated in one point with paired testicles and sperm ducts. **C**: Two testes entirely linked to two ovaries without clearly identified oviducts or sperm ducts. (ov) Ovarian tissue, (t) Testicular tissue.

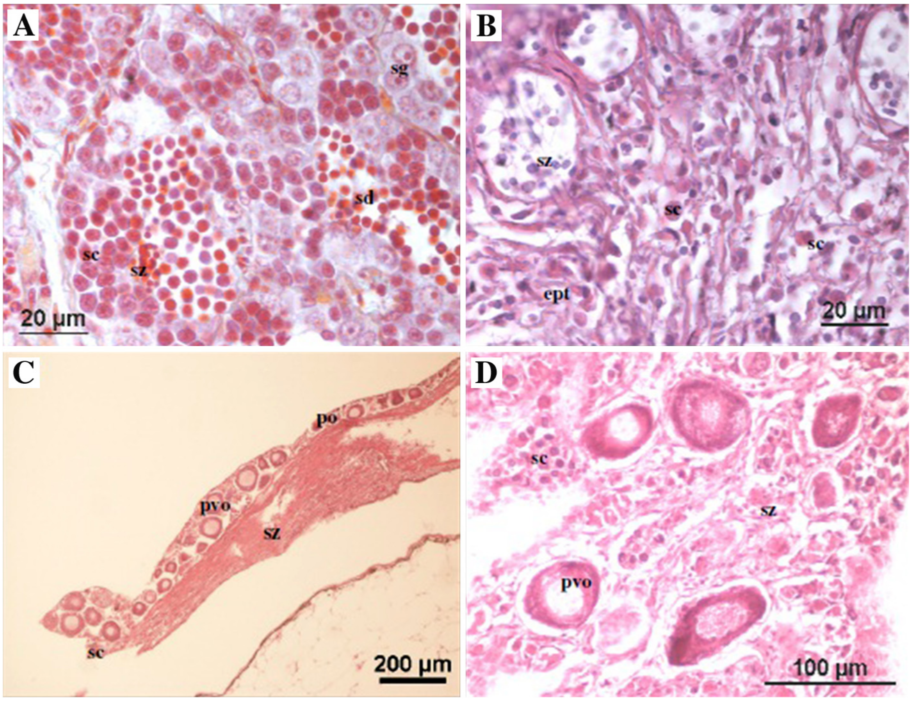

Figure 2. - Histological sections of testes in *B. callensis*. **A**: In normal males containing germinal cells at different stages of spermatogenesis and mature spermatozoa. B, C, D: In male portion of intersex individuals. **B**: Epithelial tissue accompanied by a small amount of spermatozoa and spermatocytes. **C**: Primary and previtellogenic oocytes are located in the periphery and/or in clusters within the gonad. **D**: Previtellogenic oocytes scattered throughout the testicular tissue. (ept) Epithelial tissue, (po) Primary oocytes, (pvo) Previtellogenic oocytes, (sg) Spermatogonia, (sc) Spermatocyte, (sd) Spermatide, (sz) Spermatozoa. Staining techniques: (A) Modified azan, (B, C, D) Mayer's haemalun-eosin.

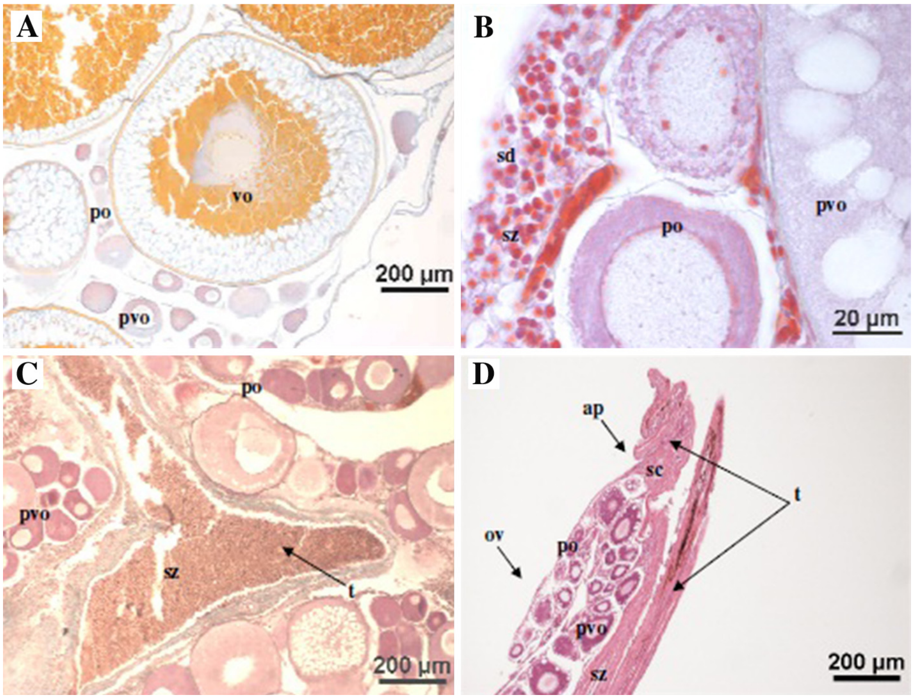

Figure 3. - Histological sections of ovaries in *B. callensis*. **A**: In normal females showing oogenesis activity with oocytes at different stages of development. **B, C, D**: In female portion of intersex individuals. **B**: Spermatides and spermatozoa are located at the periphery of the ovary. **C**: Male tissue containing a high proportion of spermatozoa interspersed within the ovary tissue. **D**: Attached portion between male and female gonads contained oocytes and spermatozoa. (po) Primary oocytes, (pvo) Previtellogenic oocytes, (vo) Vitellogenic oocytes, (sc) Spermatocyte, (sd) Spermatide, (sz) Spermatozoa, (ov) Ovarian tissue, (t) Testicular tissue, (ap) Attached portion. Staining techniques: (A, B, C) Modified azan, (D) Mayer's haemalun-eosin.

sex individuals, including date and age of captured individuals. Total length of intersex specimens (n = 16 among 700 fish sampled) ranged between 137 and 290 mm, total weight between 26.67 and 301.60 g and gonad weight between 0.36 and 11.90 g. Scales analysis revealed that the individuals were 2 to 5 years old.

Anatomical analysis of reproductive system

Macroscopic examination of the external body indicated that almost all barbels were free from important lesions and abnormalities. However, visible signs of anatomical disorders in gonads were observed, including gonad deformities, which were classified into three categories: compartmentali-

Table I. - Meristic measurements of intersex individuals, date of capture and age of fishes.

Date	Total length (mm)	Total weight (g)	Gonad weight (g)	Age (year)
29 May 2010	180	65.35	1.40	3^+
01 Jun. 2010	168	61.32	11.90	3^+
01 Jun. 2010	165	56.68	0.88	3^+
02 Aug. 2010	140	32.53	0.36	2^+
02 Aug. 2010	183	71.51	3.14	5^+
02 Aug. 2010	245	181.18	3.14	5^+
02 Aug. 2010	273	288.60	5.71	5^+
23 Oct. 2010	137	26.67	0.58	3^+
23 Oct. 2010	158	40.71	0.46	3^+
23 Oct. 2010	190	74.03	1.17	3^+
23 Oct. 2010	195	83.96	1.19	4^+
23 Oct. 2010	200	72.82	0.95	4^+
23 Oct. 2010	219	114.27	3.96	4^+
23 Oct. 2010	200	96.20	1.80	4^+
23 Oct. 2010	234	165.80	6.04	5^+
23 Oct. 2010	290	301.60	11.11	5^+

zation, intersex and constrictions, the latter more frequent in males. Through anatomical observations, the reproductive system of normal females and males consisted of a pair of ovaries and a pair of testicles, respectively.

In contrast, intersex specimens had both male and female gonads macroscopically distinct (Fig. 1). The female portion showed pink pigmentation, as in normal ovaries and included eggs clearly visible, the fully mature oocytes were released in the abdominal cavity. The male portion had nearly the same morphology as a normal testis with white pigmentation. In some cases, it contained thick milt such as in normal ripe males. This macroscopic analysis revealed the existence of three abnormal structural forms of the reproductive system. Two individuals presented only one ovary and one testis clearly separated, without oviduct or sperm ducts (Fig. 1A). Eight specimens presented paired ovaries and oviducts associated in one point with paired testicles and sperm ducts (Fig. 1B). Finally, six specimens contained two testes entirely linked to the two ovaries, without clearly identified oviducts or sperm ducts (Fig. 1C).

Histological analysis

Normal males and females, collected at the same date and on the same place, were used as histological controls of the reproductive system. In individuals showing macroscopically male gonads, the histological examination revealed a functional male reproductive system. The testes contained both germinal cells at different stages of spermatogenesis, and mature spermatozoa (Fig. 2A). Histological sections of ovaries of normal females showed oogenesis activity with oocytes at different stages of development (Fig. 3A).

However, intersex fish were characterized by simultaneous presence of both male and female gonadal features, which were arranged as an ovotestis or testis-ovo.

In the male parts of intersex specimens, histological observations revealed remarkable presence of an epithelial tissue without clearly defined lobular or cystic structures, accompanied by a small amount of spermatozoa and spermatocytes (Fig. 2B). Many clusters of basal lamellae without any clearly identified somatic cells were present. Primary and previtellogenic oocytes were located in the periphery and/or in clusters within the gonad (Fig. 2C). In addition, these male portions showed a small proportion of previtellogenic oocytes scattered throughout the testicular tissue (Fig. 2D).

Inside the female portion of intersex specimens, male and female germ cells coexisted and two basic patterns were observed. In the first pattern, the gonad appeared like a normal ovary, with male islets, containing germinal cells, located at the periphery of the gonad (Fig. 3B). The second pattern showed a high proportion of male islets interspersed within the ovary tissue (Fig. 3C). Another pattern of intersexuality consisted of an attached portion between male and female gonads containing both oocytes and spermatozoa (Fig. 3D).

DISCUSSION

Barbus callensis is a gonochoric species largely present in North Africa freshwaters. Several studies have been conducted concerning its reproduction biology (Kraiem, 1997), biogeography (Almaça, 1976; Kraiem, 1986; Almaça, 1990), diet (Cherghou *et al*., 2002), growth (Kraiem, 1989), morphometry (Kraiem, 1994; Chaouachi and Ben Hassine, 1998) and systematic (Pellegrin, 1913, 1939; Estève, 1947; Almaça, 1966, 1968, 1970, 1971, 1972; Agnèse, 1990; Doadrio, 1990; Berrebi *et al*., 1995; Kraiem, 1998; Boumaïza and Quignard, 2002). To date, no report of intersex had been signalled concerning this species, and it seems to be difficult to consider this hermaphroditism as a normal occurring.

Intersex specimens represented 2.28% of the total collected individuals; this percentage concerned only the macroscopic features of hermaphroditism, meaning the apparent simultaneous presence of male and female gonads in the same individual. This percentage is tightly closer to those reported for other fish species, Bernet *et al*. (2004), Bittner *et al*. (2009) and Hinck *et al*. (2009), reported 1.1, 1.7, and 3% of macroscopic intersex, respectively.

In the present report, intersexuality in *Barbus callensis* was manifested in a wide variety of features, including deformations (constrictions and compartmentalization) of the gonads and/or reproductive ducts with altered gamete production. However, we were unable to determine the sex derivation because the proportions of male and female gonads in the same individuals were so close to each other that it was not possible to establish whether the incidence of intersexuality was due to feminization or masculinisation. The anatomical analysis of *B. callensis* showed that male and female gonads could be present in a same individual. Nolan *et al.* (2001) reported that in all the intersexes, the most common malformation was the presence of fish with both sperm duct and ovarian cavity. In other hand, Bittner *et al.* (2009) demonstrated that the intersex and compartmentalization abnormalities in whitefish (*Coregonus* spp.) were considered like true gonad deformations. These authors reported that males showed significantly more gonad deformations than females. This is in accordance with our results, showing that constrictions are more frequent in males.

Sant'Anna *et al.* (2010) reported that the different parts of intersex gonads were unfunctional and organized as an ovotestis and testis-ovo. De Metrio *et al.* (2003) reported dissimilar findings in intersex Mediterranean swordfish *Xiphias gladius* (Linnaeus, 1758) and hypothesized that the functionality of the testes did not seem to be impaired. In the same time, Urbach *et al.* (2008) and Bittner *et al.* (2009) demonstrated that the reproductive capability of altered Whitefish (*Coregonus* spp.) were not reduced and that no difference was observed in sperm quality between normal and abnormal males. However, Jobling *et al.* (2002a), demonstrated that male gametes production and quality were reduced in intersex roach when compared to normal males collected from a reference site, intersex fish produced almost 50% less milt than did reference males. Concerning *B. callensis* intersex individuals, the general structure of the testes containing both oocytes and spermatozoa were damaged when compared with those of normal males of similar size and captured during the same period.

In *B. callensis*, female portions showed a higher incidence of oocyte atresia when compared to the reference females, 80% of the cross-sectional area of the gonad were composed by atretic oocytes. From similar observations, Jobling *et al.* (2002b) suggested that fish might have a lowered fecundity because of atresia in intersex wild female roach. In the present study, the functionality of the intersex individuals was not investigated. Further studies using computer-aided semen analysis are needed, particularly in order to analyse accurately male gametes motility according to intersex situations with concomitant measurement of sex steroid hormones and vitellogenin.

The mechanisms underlying sex determination and differentiation in fish are variable in response to environmental parameters (Baroiller, 1998). In fish, sex-reversal, particularly in gonochoric species, seems to be widely linked to pollution (Jones and Reynolds, 1997; Parks *et al.*, 2001; Van Aerle *et al.*, 2001; Lokman *et al.*, 2002; Gibson *et al.*, 2005; Kipfer *et al.*, 2009; Prado *et al.*, 2011; Sanchez *et al.*, 2011; Baynes *et al.*, 2012; Blazer *et al.*, 2012).

Concerning Soummam River, Mouni *et al.* (2009) reported that numerous sources of pollution were present. Thus, dozens of plants activities have been noted, with also 58 oil mills, 33 washing greasing stations and 26 public dumps. A volume of 165.10^5 m^3 of untreated urban wasted water is released annually. All these factors induce a huge pollution, with BOD_5 (biological oxygen demand in five days) and COD (chemical oxygen demand) values reaching 114.3 mg/L and 196.56 mg/L, respectively. These two parameters are considered as real indicators of water quality, the maximum accepted values are 25 mg/L and 40 mg/L for BOD_5 and COD respectively (Mouni *et al.*, 2009).

Studies conducted in recent decades suggested that contact with anthropogenic chemicals and mimics of estrogens and testosterone might alter sexual development in many vertebrate groups, including fishes (Kime, 1999; Iguchi *et al.*, 2007; Williams *et al.*, 2009). It was particularly established that endocrine-disrupting compounds can alter gonad morphology in fish (Allen *et al.*, 1999; Van der Ven *et al.*, 2003; Vethaak *et al.*, 2005; Schäfers *et al.*, 2007). Barnhoorn *et al.* (2010) have recently demonstrated that exposure of wild fish to non-treated sewage effluents caused sex reversal in a freshwater fish species *Oreochromis mossambicus* in South Africa. These authors reported that 49% of testes contained oocytes. Bernet *et al.* (2008) hypothesized that the probable cause of gonadal deformities was due to the accumulation of xenobiotics in whitefish through the plankton.

Different molecules are suspected to be linked to intersex alteration, Bernhardt *et al.* (2006) and Bernhardt and Hippel (2008), using the threespine stickleback *Gasterosteus aculeatus* (Linnaeus, 1758), demonstrated that perchlorate could produce androgenic effects, and some females were masculinised to the extent that they produced both sperm and eggs. Iguchi *et al.* (2007) reported that trenbolone at 1 μg/L induced spermatocytes apparition in the ovary of sexually mature females and the contraceptive estrogen ethinylestradiol induced feminised responses in the medaka *Oryzias latipes* (Temminck & Schlegel, 1846). In The Netherlands, Lahr *et al.* (2006) signalled that high concentrations of vitellogenin were found in the freshwater bream *Abramis brama* and in the estuarine flounder *Platichthys flesus* (Linnaeus, 1758) related to estrogenic compounds inducing male feminization. In a number of British rivers contaminated with sewage effluent, 100% of sampled males of roach *Rutilus rutilus* fish presented testes producing simultaneously male and female gametes (Jobling *et al.*, 1998; Beresford *et al.*, 2004).

In mammals, it has been shown that reproductive defects caused by endocrine disruptors, including estrogenic compounds, can be inherited by future generations epigenetically (Anway *et al.*, 2005; Crews *et al.*, 2007). In fish, inheritance of gonad deformations (Bittner *et al.,* 2011) and improper breeding in hatchery (Bernet *et al.,* 2004) as intersex factors have been excluded. Brown *et al.* (2009) demonstrated that when considering diminishing offspring survival as a specific reproductive defect in rainbow trout, no heritable effect was observed from the F1 generation. In addition, when contrasting gene expression profiling of male whitefish with normal and deformed gonads, only weak differences have been observed (Bittner *et al*., 2011). As consequence of all these findings, a primary genetic pathway as a causative effect in intersex remains very likely.

The causative agent(s) of intersexuality in *Barbus callensis* living in Soummam River affected by combined domestic and industrial sewage effluent are unknown. We hypothesized that this phenomenon was caused by exposure to mixtures of contaminants interfering with reproductive differentiation and development. Moreover, when looking at the total length of intersex individuals, the minimal observed value was 137 mm, which probably means that intersex appears after a period of exposure to the contaminants. The Soummam valley is characterized by the presence of a large number of mills that discharge olive oil mill wastewaters (OMW) into Soummam River without previous treatment. The OMW is considered as polluting effluent which may degrade the water quality, with critical negative impacts on ecosystems, this impact particularly on aquatic species, has been largely reviewed by Justino *et al*. (2012). The OMW are known to contain polyphenols presenting similarities with sexual steroids, including estrogens and androgens that can affect intersex in fish species.

This study emphasizes the need for a multi-parameters approach in detecting endocrine disrupting chemicals, particularly the estrogenic and/or androgenic mimics. In parallel, it is of high importance to establish whether the incidence of intersexuality was due to feminization or masculinisation by determining the genetic sex of hermaphroditism specimens and by assaying steroid hormones and vitellogenin.

REFERENCES

AGNÈSE J.F., BERREBI P., LÉVÊQUE O. & GUÉGAN J.F., 1990. - Two lineages, diploid and tetraploid, demonstrated in African species of *Barbus* (Osteichthyes, Cyprinidae). *Aquat. Living. Resour.,* 3: 305-311.

ALLEN Y., SCOTT A.P., MATTHIESSEN P., HAWORTH S., THAIN J.E. & FEIST S., 1999. - Survey of estrogenic activity in United Kingdom estuarine and coastal waters and its effects on gonadal development of the flounder *Platichthys flesus*. *Environ. Toxicol. Chem*., 18: 1791-1800.

ALMAÇA C., 1966. - Sur la systématique des Barbeaux marocains (Pisces, Cyprinidae, *Barbus*). *Arq. Mus. Boc*., 1: 111-121.

ALMAÇA C., 1968. - Révision critique de quelques types de Cyprinidés d'Europe et d'Afrique du Nord des collections du Muséum national d'Histoire naturelle. *Bull. Mus. Natl. His. Nat*., 40: 1116-1144.

ALMAÇA C., 1970. - Sur les Barbeaux (genre et sous-genre *Barbus*) de l'Afrique du Nord. *Bull. Mus. Natl. Hist. Nat*., 42: 141-158.

ALMAÇA C., 1971. - Sur la spéciation des Barbeaux Nord-Africains. *Bull. Mus. Natl. Hist. Nat.,* 42: 853-856.

ALMAÇA C., 1972. - Sur la systématique des Barbeaux (genre et sous-genres *Barbus*) de la péninsule Ibérique et de l'Afrique du Nord. *Arch. Mus. Boc*., 3: 319-346.

ALMAÇA C., 1976. - Remarks on the biogeography of Euro-Mediterranean *Barbus* (Cyprinidae, Pisces). *Bull. Ecol*., 19: 23-30.

ALMAÇA C., 1990. - Neogene Circum-Mediterranea paleogeography and Euro-Mediterranean *Barbus* biogeography. *Arq. Mus. Boc*., 4: 585-611.

ANWAY M.D., CUPP A.S., UZUMCU M. & SKINNER M.K., 2005. - Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science*, 308: 1466-1469.

BAGLINIÈRE J.L., SABATIÉ M.R., APRAHAMIAN M.W. *et al.* [12 authors], 2001. - Guide pour l'interprétation des écailles et l'estimation de l'âge chez les aloses (*Alosa* spp.) de la façade atlantique-est et de la Méditerranée-ouest. *Bull. Fr. Pêche Piscic*., 357/360: 485-527.

BARNHOORN I.E.J., VANDYK J.C., PIETERSE G.M. & BORNMAN M.C., 2010. - Intersex in feral indigenous freshwater *Oreochromis mossambicus*, from various parts in the Luvuvhu River, Limpopo Province, South Africa. *Ecotoxicol. Environ. Saf.*, 73(7):1537-1542.

BAROILLER J.F., 1998. - Le déterminisme environnemental du sexe chez les poissons gonochoriques. *Piscic. Fr.*, 133: 51-59.

BAYNES A., GREEN C., NICOL E., BERESFORD N., KANDA R., HENSHAW A., CHURCHLEY J. & JOBLING S., 2012. - Additional treatment of wastewater reduces endocrine disruption in wild fish – a comparative study of tertiary and advanced treatments. *Environ. Sci. Technol*., 46: 5565-5573.

BERESFORD N., JOBLING S., WILLIAMS R. & SUMPTER J.P., 2004. - Endocrine disruption in juvenile roach from English rivers: a preliminary study. *J. Fish. Biol*., 64: 580-586.

BERNET D., WAHLI T., KUENG C. & SEGNER H., 2004. - Frequent and unexplained gonadal abnormalities in whitefish (central alpine *Coregonus* sp.) from an alpine oligotrophic lake in Switzerland. *Dis. Aquat. Org*., 61: 137-148.

BERNET D., LIEDTKE A., BITTNER D., EGGEN R.I.L., KIPFER S., KUNG C., LARGIADER C.R., SUTER M.J.F., WAHLI T. & SEGNER H., 2008. - Gonadal malformations in whitefish from Lake Thun: defining the case and evaluating the role of EDCs. *Chimia*, 62: 383-388.

BERNHARDT R.R. & VON HIPPEL F.A., 2008. - Chronic perchlorate exposure impairs stickleback reproductive behaviour and swimming performance. *Behaviour*, 145: 527-559.

BERNHARDT R.R., VON HIPPEL F.A. & CRESKO W.A., 2006. - Perchlorate induces hermaphroditism in threespine Sticklebacks. *Environ. Toxicol. Chem*., 25: 2087-2096.

BERREBI G.P., KRAIEM M.M., DOADRIO I., GHARBI S.E.L. & CATTANEO-BERREBI G., 1995. - Ecological and genetic differentiation of *Barbus callensis* populations in Tunisia. *J. Fish. Biol*., 47: 850-864.

BITTNER D., BERNET D., WAHLI T., SEGNER H., KUNG C. & LARGIADE C.R., 2009. - How normal is abnormal? Discrimination between deformations and natural variation in gonad morphology of European whitefish *Coregonus lavaretus*. *J. Fish. Biol*., 74: 1-21.

BITTNER D., COSSINS A.R., SEGNER H., EXCOFFIER L. & LARGIADÈR C.R., 2011. - Identification of candidate genes and physiological pathways involved in gonad deformation in whitefish (*Coregonus* spp.) from Lake Thun, Switzerland. *Int. J. Environ. Res. Public Health*, 8: 2706-2733.

BLAZER V.S., IWANOWICZ L.R., HENDERSON H., MAZIK P.M., JENKINS J.A., ALVAREZ D.A. & YOUNG J.A., 2012. - Reproductive endocrine disruption in smallmouth bass (*Micropterus dolomieu*) in the Potomac River basin: spatial and temporal comparisons of biological effects. *Environ. Monit. Assess.*, 184: 4309-4334.

BOUMAÏZA M. & QUIGNARD J.P., 2002. - Sur la systématique des poissons des eaux continentales de Tunisie. *Rev. FSB*, 1: 174-199.

BROWN K.H., SCHULTZ I.R. & NAGLER J.J., 2009. - Lack of a heritable reproductive defect in the offspring of male rainbow trout exposed to the environmental estrogen 17α-ethynylestradiol. *Aquat. Toxicol.*, 91: 71-74.

CHAOUACHI B. & BEN HASSINE O.K., 1998. - Le barbeau *Barbus callensis* Valenciennes, 1842 (Poisson, Téléostéen) de la lagune de l'Ichkeul. *Arch. Inst. Pasteur. Tunis*, 75: 211-218.

CHARNIAUX-COTTON H., 1975. - Hermaphroditism and gynandromorphism in malacostracan Crustacea. *In*: Intersexuality in the Animal Kingdom (Reinboth R., ed.), pp. 91-105. Berlin: Springer-Verlag.

CHERGHOU S., KHODARI M., YAÂKOUBI F., BENABID M. & BADRIN A., 2002. - Contribution to study a barbel (*Barbus barbus callensis* Valenciennes, 1842) diet in Moroccan Medium-Atlas stream: Oued Boufekrane. *Rev. Sci. Eau*, 15: 153-163.

CREWS D., GORE A.C., HSU T.S., DANGLEBEN M.L., SPINETTA M., SCHALLERT T., ANWAY M.D. & SKINNER M.K., 2007. - Transgenerational epigenetic imprints on mate preferences. *Proc. Natl. Acad. Sci. USA*, 104: 5942-5946.

DE METRIO G., CORRIERO A., DESANTIS S. *et al.* [11 authors], 2003. - Evidence of a high percentage of intersex in the Mediterranean swordfish (*Xiphias gladius* L.). *Mar. Poll. Bull.*, 46: 358-361.

DOADRIO I., 1990. - Phylogenetic relationships and classification of west palaearctic species of the genus *Barbus* (Osteichthyes, Cyprinidae). *Aquat. Living. Resour.,* 3: 265-282.

ESTÈVE R., 1947. - Étude biométrique des barbeaux marocains. *Bull. Mus. Natl. Hist. Nat.*, 19: 265-270.

GIBSON R., SMITH M.D., SPARY C.J., TYLER C.R. & HILL E.M., 2005. - Mixtures of estrogenic contaminants in bile of fish exposed to wastewater treatment. Works effluents. *Environ. Sci. Technol.*, 39: 2461-2471.

HINCK J.E., BLAZER V.S., SCHMITT C.J., PAPOULIAS D.M. & TILLITT D.E., 2009. - Widespread occurrence of intersex in black basses (*Micropterus* spp.) from US rivers. *Aquat. Toxicol.*, 95: 60-70.

IGUCHI T., KATSU Y., URUSHITANI H., LANGE A. & Tyler C.R., 2007. - Developmental reproductive effects of exposure to pharmaceutical steroids in the aquatic environment: studies on Mosquitofish (*Gambusia affinis affinis*), Roach (*Rutilus rutilus*) and Medaka (*Oryzias latipes*). *J. Mar. Sci. Technol.*, 15 (special issue): 29-36.

JAFRI S.I.H. & ENSOR D.M., 1979. - Occurrence of an intersex condition in the roach *Rutilus rutilus* L. *J. Fish. Biol.*, 14: 547-549.

JOBLING S., NOLAN M., TYLER C.R., BRIGHTY G. & SUMPTER J.P., 1998. - Widespread sexual disruption in wild fish. *Environ. Sci. Technol.*, 32: 2498-2506.

JOBLING S., COEY S., WHITMORE J.G. *et al.* [11 authors], 2002a. - Wild intersex roach (*Rutilus rutilus*) have reduced fertility. *Biol. Rep.*, 67: 515-524.

JOBLING S., BERESFORD N., NOLAN M., RODGERS-GRAY T., BRIGHTY G.C., SUMPTER J.P. & TYLER C.R., 2002b. - Altered sexual maturation and gamete production in wild roach (*Rutilus rutilus*) living in rivers that receive treated sewage effluents. *Biol. Reprod.*, 66: 272-281.

JONES J.C. & REYNOLDS J.D., 1997. - Effects of pollution on reproductive behavior of fishes. *J. Fish. Biol.*, 7: 463-491.

JUSTINO C.I.L., PEREIRA R., FREITAS A.C., ROCHA-SANTOS T.A.P., PANTELEITCHOUK T.S.L. & DUARTE A.C., 2012. - Olive oil mill wastewaters before and after treatment: a critical review from the ecotoxicological point of view. *Ecotoxicology*, 21: 615-629.

KIME D.E., 1999. - A strategy for assessing the effects of xenobiotics on fish reproduction. *Sci. Total. Environ.*, 225: 3-11.

KIPFER S., SEGNER H., WENGER M., WAHLI T. & BERNET D., 2009. - Long-term estrogen exposure of whitefish *Coregonus lavaretus* induces intersex but not Lake Thun-typical gonad malformations. *Dis. Aquat. Organ.*, 84: 43-56.

KRAIEM M.M., 1986. - Influence de la salinité sur la présence des barbeaux *Barbus callensis* Valenciennes, 1842 (Pisces, Cyprinidae) dans le lac Ichkeul (Tunisie septentrionale). *Bull. Inst. Natl. Sci. Tech. Océanogr. Pêche Salammbô*, 13: 89-94.

KRAIEM M.M., 1989. - Étude comparée de la croissance de différentes populations de *Barbus callensis* Valenciennes 1842 (Pisces, Cyprinidae) de Tunisie. *Cybium*, 13: 365-374.

KRAIEM M.M., 1994. - Analyse de la variabilité morphométrique et méristique des populations de *Barbus callensis* Valenciennes, 1842 (Poissons, Cyprinidés) de Tunisie. *Bull. Fr. Pêche Piscic.*, 334: 201-212.

KRAIEM M.M., 1997. - Chronologie de la reproduction et cycle de développement des gonades chez *Barbus callensis* Valenciennes, 1842 (Pisces, Cyprinidae) de Tunisie. *Bull. Inst. Natl. Sci. Technol. Mer*, 24: 74-88.

KRAIEM M.M., 1998. - Étude synthétique de la systématique et de la biogéographie de *Barbus* Cuvier, 1817 (Pisces, Cyprinidae). Cas des barbeaux tunisiens. *Bull. Inst. Natl. Sci. Technol. Mer*, 25: 101-117.

LAHR J., KUIPER R.V., VAN MULLEM A. *et al.* [12 authors], 2006. - A field survey of estrogenic effects in freshwater and marine fish in the Netherlands. *Soc. Environ. Toxicol. Chem.*, 6: 151-178.

LOKMAN P.M., HARRIS B., KUSAKABE M., KIME D.E., SCHULZ R.W., ADACHI S. & YOUNGA G., 2002. - 11-Oxygenated androgens in female teleosts: prevalence, abundance, and life history implications. *Gen. Comp. Endocrinol.*, 129 : 1-12.

MOUNI L., MERABET D., ARKOUB H. & MOUSSACEB K., 2009. - Étude et caractérisation physico-chimique des eaux de l'oued Soummam (Algérie). *Sécheresse*, 20: 360-366.

NOLAN M., JOBLING S., BRIGHTY G., SUMPTER J.P. & TYLER C.R., 2001. - A histological description of intersexuality in the roach. *J. Fish. Biol.*, 58: 160-176.

PARKS L.G., LAMBRIGHT C.S., ORLANDO E.F., GUILLETTE L.J.J., ANKLEY G.T. & GRAY L.E.J., 2001. - Masculinization of female mosquitofish in kraft mill effluent-contaminated fenholloway river water is associated with androgen receptor agonist activity. *Toxicol. Sci.*, 62: 257-267.

PELLEGRIN J., 1913. - Sur une variété nouvelle d'Algérie et de Tunisie de *Barbus callensis* C. V. provenant de l'oasis de Figuig (Maroc). *Bull. Soc. Zool. Fr.,* 38: 119-120.

PELLEGRIN J., 1939. - Les barbeaux de l'Afrique du Nord française : description d'une espèce nouvelle. *Bull. Soc. Sci. Nat. Maroc*, 19: 1-10.

PRADO P.S., SOUZA C.C., BAZZOLI N. & RIZZO E., 2011. - Reproductive disruption in lambari *Astyanax fasciatus* from a Southeastern Brazilian reservoir. *Ecotoxicol. Environ. Saf.*, 74: 1879-1887.

PURDOM C.E., HARDIMAN P.A., BYE V.J., ENO N.C., TYLER C.R. & SUMPTER J.P., 1994. - Estrogenic effects of effluent from sewage treatment works. *Chem. Ecol.*, 8: 275-285.

SANCHEZ W., SREMSKI W., PICCINI B. *et al.* [12 authors], 2011. - Adverse effects in wild fish living downstream from pharmaceutical manufacture discharges. *Environ. Int.*, 37: 1342-1348.

SANT'ANNA B.S., TURRA A. & ZARA F.J., 2010. - Simultaneous activity of male and female gonads in intersex hermit crabs. *Aquat. Biol.*, 10: 201-209.

SCHÄFERS C., TEIGELER M., WENZEL A., MAACK G., FENSKE M. & SEGNER H., 2007. - Concentration- and time-dependent effects of the synthetic estrogen, 17alpha-ethinylestradiol, on reproductive capabilities of the zebrafish, *Danio rerio*. *J. Toxicol. Environ. Health. A.*, 70: 768-779.

URBACH D., BRITSCHGI A. & JACOB A., 2008. - Gonadal alterations in male whitefish *Coregonus fatioi*: no evidence for genetic damage reducing viability in early life stages. *Dis. Aquat. Organ.*, 81: 119-125.

VAN AERLE R., NOLAN M., JOBLING S., CHRISTIANSEN L.B., SUMPTER J.P. & TYLER C.R., 2001. - Sexual disruption in a second species of wild cyprinid fish (the gudgeon, *Gobio gobio*) in United Kingdom freshwaters. *Environ. Toxicol. Chem.*, 20: 2841-2847.

VAN DER VEN L.T., WESTER P.W. & VOS J.G., 2003. - Histopathology as a tool for the evaluation of endocrine disruption in zebrafish (*Danio rerio*). *Environ. Toxicol. Chem.*, 22: 908-913.

VETHAAK A.D., LAHR J., SCHRAP S.M. *et al.* [15 authors], 2005. - An integrated assessment of estrogenic contamination and biological effects in the aquatic environment of The Netherlands. *Chemosphere*, 59: 511-524.

VIGANÒ L., ARILLO A., BOTTERO S., MASSARI A. & MANDICH A., 2001. - First observation of intersex cyprinids in the Po River (Italy). *Sci. Total. Environ.*, 269: 189-194.

WILLIAMS R.J., KELLER V.D.J., JOHNSON A.C. & YOUNG A.R., 2009. - A national risk assessment for intersex in fish arising from steroid estrogens. *Environ. Toxicol. Chem.*, 28: 220-230.

Reçu le 19 mai 2012.
Accepté pour publication le 17 décembre 2012.